STUDIES ON EPITHELIAL GLYCOPROTEINS

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Almqvist & Wiksells Boktryckeri AB

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Studies on Epithelial Glycoproteins

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IVAR WERNER

This paper is a summary of the following three communications:

I. Werner, I.: The Identification of Small Amounts of Sugars by Means of X-ray Diffraction Patterns, *Mikrochemie 39*, 133, 1952.
II. Werner, I. and Odin, L.: On the Presence of Sialic Acid in Certain Glycoproteins and in Gangliosides, *Acta Soc. Med. Upsal. 57*, 230, 1952.
III. Werner, I.: Studies on Glycoproteins from Mucous Epithelium and Epithelial Secretions, *Acta Soc. Med. Upsal. 58*, 1, 1953.

References to these papers will be made with Roman figures I—III.

The mucous epithelium and secretions of higher animals have for long been known to have a high glycoprotein content. Very little, however, is known about the chemistry of these glycoproteins and in many cases only old and incomplete chemical data are available.

Thus, for instance, the glycoproteins of the respiratory tract have not been subjected to a detailed chemical investigation since the work of Fr. Müller in 1901 (1). Müller was able to prepare from expectorated sputa glycoproteins containing 20—35 % carbohydrate. Among the constituents he identified glucosamine and also obtained an osazone which was probably galactosazone.

The mucous glycoproteins of the digestive tract are somewhat better known. In 1916—1918 Levene and Lopez-Suarez (2, 3) reported the isolation from pig stomach mucosa of an acid polysaccharide which was called mucoitinsulphuric acid. The acid contained acetylglucosamine, glucuronic acid, and esterified sulphuric acid, which were presumed to be present in equimolecular amounts. The final evidence of this composition, however, was not established until the works of Meyer *et al.* (4) and Wolfrom & Rice (5) were published in 1937 and 1947 respectively.

Meyer *et al.* (4) were also able to show that mucoitinsulphuric acid constituted a small part only of the total glycoprotein content of the

gastric mucosa. The glycoprotein was found to consist mainly of a neutral carbohydrate complex, among the constituents of which they identified glucosamine and galactose. The glycoprotein also showed blood group activity.

It has long been known that many epithelial mucosae and secretions exhibit blood group activity (see 6). Highly active preparations have also been isolated from several such sources, e. g. stomach mucosa, ovarian cysts, saliva, etc. All blood group substances so far investigated are glycoproteins containing glucosamine, chondrosamine, galactose, and fucose (for references see 7, 8, 9). The serologically active substances, however, in most cases constitute only a minor part of the total glycoprotein material.

In 1936 Blix (10) showed that the main component of submaxillary mucin is a glycoprotein containing a nitrogenous, reducing acid of disaccharidic nature, later called sialic acid (11). Besides this acid the glycoprotein contained chondrosamine (11).

The mucous secretion from cervix uteri has recently been examined by Shettles *et al.* (12) and by Bergman & Werner (13). Both groups of workers found hexosamine, hexose, and methylpentose as glycoprotein constituents. The hexose was shown chromatographically to be galactose, and the methylpentose to be fucose (13).

The aim of the present investigation has been to learn more about the chemistry of epithelial mucus from different organs. The main object has been to determine qualitatively and quantitatively the simple sugars occurring in the glycoproteins of mucous epithelium and secretions, and find out to which main types of glycoproteins these sugars belong. No attempt, however, has been made to isolate the different glycoproteins in the native state.

It was clear from the outset that various technical difficulties would be met with, and a considerable part of the investigation had to be devoted to the study of analytical and preparative methods.

INVESTIGATIONS

ANALYTICAL METHODS (I, II, III)

The quantitative determinations of hexosamine, hexoses, methylpentose, hexuronic acid, organically bound sulphate, and nitrogen, were carried out according to earlier known methods. These methods have been critically evaluated with respect to their accuracy and some difficulties in the original procedures (III). In a few cases minor technical modifications have been introduced.

Early in the work it became evident that the mucus contained substances giving the colour reactions typical of sialic acid, i. e. the 'direct'

Ehrlich reaction, and the violet Bial recation (10, 14). The specificity of these reactions had not been studied before and this had to be done. Further, it was necessary to work out a method for the quantitative determination of sialic acid. These problems were subjected to a special study (II). Both the Bial and the Ehrlich reagents were found to give colour reactions of sufficient specificity for the present purposes. Thus two fairly accurate, quantitative methods were worked out and described in detail. It was further observed that sialic acid also formed strong and characteristic colours with the diphenylamine reagent of Dische (15, 16) and the tryptophane-perchloric acid reagent (17, 18). These two reagents may also be used for quantitative purposes but in some respects they are inferior to those mentioned above.

By means of the colour reactions and procedures known to liberate carbon dioxide from sialic acid, results were obtained which strongly indicated the presence of sialic acid or some very closely related compound in serum glycoproteins, ovomucin (II), and mucous secretion (III).

Another problem was to find out a suitable method of identifying the monosaccharide constituents of glycoproteins. The routine qualitative analyses of sugars and amino acids were carried out by paper partition chromatography. Many substances, however, are very difficult to identify even with this technique and, of course, the demonstration of a coloured spot on a paper strip is no absolute evidence of the identity of a substance. Working with biological material the amounts available are often small and the substances impure, and in the case of sugars many of them give very similar chemical recations. Thus identification by ordinary chemical methods is often impossible.

An excellent means of identifying small amounts of free sugars was found to be the X-ray powder diagrams, but these had previously been described for only a few of the sugars known to appear in animal tissue, and most of them had to be determined, which required the preparation of the sugars in a sufficiently pure, crystalline state. Thus the powder diagrams of the following sugars were determined: α- and β-D-glucose and the glucose monohydrate; α- and β-D-galactose; α- and β-D-mannose; D-fructose; D-glucuronolactone; α- and β-D-glucosamine hydrochloride; α- and β-D-chondrosamine hydrochloride; α-L-fucose; D-ribose; β-D-arabinose; β-D-lyxose; and α-D-xylose (I).

RESULTS (III)

Respiratory tract

Nasal Mucus

Several samples of human nasal mucus were examined. The organic matter of the mucus was found to be a glycoprotein mixture containing

10—30 % of carbohydrate. All samples contained hexosamine, galactose, fucose, and sialic acid, and some, especially those from cases of purulent rhinitis, also contained small amounts of mannose. There was no sign of the presence of hexuronic acid.

Amino acid chromatography showed the presence of all the ordinary amino acids.

There are apparently two forms of nasal mucus. They differ in physical appearance but have the same chemical composition, one being a viscous liquid, the other a soft gel. Very often the actual secretion is a mixture of both forms. The gel-mucus cannot be transformed to the liquid state by treatment with water or saline at physiological temperature. It will dissolve in weak alkali and in molar solutions of ammonium thiocyanate or potassium iodide. Both forms are decomposed at room temperature by strong alkali or mineral acids.

Treatment with proteolytic enzymes rapidly degrades the nasal mucus, giving solutions of low viscosity.

Tracheo-bronchial Mucosa and Secretion

The material consisted of mucosal scrapings from pig trachea and a few samples of expectorated sputum from a person suffering from acute bronchitis.

The pig material was digested with proteolytic enzymes and dialysed. The undialysable residue was found to contain much glycoprotein material. The monosaccharide constituents of the glycoprotein were hexosamine, galactose, fucose, and mannose. The amounts of mannose were very small compared to the other sugars. The molecular ratio hexosamine/galactose + fucose was approximately 1 : 1.

After acid hydrolysis of the digested material the hexosamine component was obtained in crystalline form as hydrochloride. Both the glucosamine and chondrosamine salts were identified by X-ray analysis.

The human sputa washed as free from saliva as possible and dried with alcohol, acetone, and ether, contained about 20 % of glycoprotein carbohydrate. Here, too, the sugar constituents were hexosamine, galactose, fucose, and sialic acid. Faint traces of mannose were also found on the chromatograms. The amino acid pattern showed no unusual features.

Digestive Tract

The material comprised mucosal scrapings from different parts of the digestive tract of pigs, the gastric secretion of a dog, human gastric (fasting) secretion, samples of the mucous secretion from human small and large intestine, and bile.

All were found to contain glycoproteins. Several methods of con-

centrating the carbohydrate components of the pig material were tested. A procedure involving thorough proteolytic digestion and dialysis followed by fractional precipitation with ethanol was found to give the most satisfactory results. During this treatment the sialic acid and most of the mannose, together with small amounts of hexosamine and galactose, are lost. The bulk of the carbohydrate, however, remains undialysable, and is obtained in the fractions precipitated by ethanol at concentrations over 50 %.

The results obtained for the different organs are given below.

The Stomach

The chief glycoprotein of pig gastric mucosa is composed of a carbohydrate part containing glucosamine, chondrosamine, galactose, and fucose, and a peptide part containing several amino acids. The peptide is very resistant to proteolysis and even after thorough digestion and fractionation makes up 20—30 % of the glycoprotein.

Hexosamine seems to constitute half the amount of carbohydrate, the other half being composed of galactose and fucose in varying proportions.

The identity of the hexosamines was established by the powder diagrams after isolation of the hydrochlorides in crystalline form.

The amino acid composition of the peptide residue is rather unusual. There is no, or very little, methionine present, and small quantities only of the cyclic amino acids. The amounts of serine and threonine are remarkably large. Similar observations have also been reported for blood group substances (19, 20).

Between 10 and 20 % of the total carbohydrate content in the mucosal lining is composed of an acid polysaccharide, apparently identical with the mucoitinsulphuric acid described by Meyer *et al.* (4) and Wolfrom (5). The acid was isolated in small amounts and the analytical data are in keeping with those reported by these earlier authors. The electrophoretical mobility of the acid was found to be very similar to that of chondroitinsulphuric acid.

It was observed that practically all mucoitinsulphuric acid derived from the 'corpus—fundus', the mucosa of the 'canalis' containing very little. It was further noted that the relative fucose content in the glycoprotein was lower in the canalis preparations than in the corresponding preparations from the corpus.

Both the glycoprotein carbohydrates were also found in human and dog gastric secretion, together with sialic acid.

The Intestine

The bulk of the carbohydrate material in the mucosal scrapings contained glucosamine, chondrosamine, galactose, and fucose. The

hexosamines constituted about half the amount of carbohydrate. These and fucose were isolated in crystalline form from both the small and large intestine.

The fractions richest in carbohydrate still contained 20—40 % of amino acids. Here, too, the contents of methionine, tyrosine, and phenylalanine, were low, and the threonine and serine contents high.

The glycoprotein fractions from intestinal mucosa thus have mainly the same composition as the stomach preparations, but it was much more difficult to concentrate the carbohydrate in the material from the small intestine and here additional purification procedures had to be used.

The presence of mucoitinsulphuric acid or some similar substance also in intestinal mucosa was strongly indicated by the analytical findings. Thus approximately equimolecular amounts of hexuronic acid and esterified sulphuric acid were found. Further, on some of the electrophoretic diagrams a small boundary was observed, which had the mobility of mucoitinsulphuric acid.

Samples of mucous secretion from human ileum and colon were found to contain 20—30 % of carbohydrate, consisting of hexosamine (10—15 %), galactose, fucose, and sialic acid.

The Gall-bladder

The colourless, mucous contents of the gall-bladder from a patient operated on for hydrops due to stone in the cystic duct were investigated. The main organic constituent was found to be a glycoprotein mixture containing about 60 % of carbohydrate, viz. hexosamine (about 24 %), galactose, fucose, and sialic acid. There were also traces of mannose.

Hexosamine, galactose, and fucose, could also be demonstrated in preparations from bovine and pig bile after acid hydrolysis.

The Female Genital Tract

The Fallopian Tubes

Mucosal scrapings from human oviducts were found to contain hexosamine, galactose, fucose, and sialic acid. Small amounts of hexuronic acid were also indicated colorimetrically.

Cervix Uteri

Normal cervical mucus, and mucous plugs of pregnancy, were investigated.

Both had a closely similar qualitative composition, containing hexosamine, galactose, fucose, and sialic acid, and the ordinary amino acids except methionine. The mutual ratio between the sugar components was also approximately the same. However, whereas the protein material of normal mucus contains about 15 % carbohydrate, that of the mucous plugs contains about 45 %. Probably the main difference between the two forms of secretion is that the plugs are nearly pure mucus, the normal secretion being mixed with other protein material.

The mucous plug is a soft gel, practically insoluble in water or saline at 37° C within a pH range of 6—10. The gel structure, however, is rapidly destroyed by proteolytic digestion.

The hexosamine component of the mucous plugs was isolated, and both glucosamine and chondrosamine identified by their X-ray diffraction patterns.

GENERAL CONCLUSIONS

There are evidently three main types of glycoprotein to be found in mucous epithelium and its secretions.

By far the most predominant type is characterized by a carbohydrate part containing glucosamine, chondrosamine, galactose, and fucose, firmly linked to a peptide group. The hexosamines seem regularly to constitute half the amount of carbohydrate. The contents of the other sugars, however is not constant. Thus the ratio fucose : hexosamine in the pig mucosal material varied between 1 : 2 and 1 : 8. In the human secretions the variation was only 1 : 2—1 : 3. Following Blix's suggestion (21), this form of carbohydrate has been named fucomucan.

The substances responsible for the blood group characters belong to this main type of glycoproteins.

A second kind of epithelial glycoprotein contains sialic acid as its characteristic carbohydrate component. In a reasonably pure state this type of glycoprotein has so far been isolated only from submaxillary mucin (10, 11), some ovarian cysts, and from the ascitic fluid of a cancer patient (unpublished data). All sialoproteins so far known contain hexosamine.

All mucous secretions studied in the present work contained sialic acid in protein combination. However, the sialic-acid-containing glycoproteins from these sources have not yet been isolated in a pure state.

The third type of epithelial glycoprotein contains mucoitinsulphuric acid. This acid is different to the other kinds of prosthetic carbohydrate in being bound more loosely to protein; small amounts might also exist in free form in the secretions. In a pure state mucoitinsulphuric acid has so far been isolated from the stomach only, but there are also indications of its presence elsewhere at least in intestinal mucosa.

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